Studies on Influenza Virus Transmission between Ferrets: the Public Health Risks Revisited

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Let’sitch and Inglesby recently estimated the potential public health risks associated with research on influenza virus transmission via respiratory droplets or aerosols between ferrets, leading them to conclude that such research is too risky to be conducted (1). The authors of that and other publications (2–4) estimated the probability of laboratory-acquired infections (LAIs) and onward transmission of the viruses under investigation, as well as the potential consequence to public health if such events were to occur. Given the weight assigned to these risk estimates, it is important that potential pitfalls in the underlying assumptions in these analyses be rigorously scrutinized. Importantly, the published estimates were based on historical data and did not take into account the numerous risk reduction measures that are in place in the laboratories where the research is conducted. Here, I provide a critical appraisal of the published work, discussing, challenging, and modifying the estimates based on the specific conditions under which the work is performed and the properties of the viruses under investigation. By doing so, the outcome of the risk assessment changes from serious risks to negligible risks for humans and the environment. As a consequence, a more balanced debate about the research on influenza virus transmission via respiratory droplets or aerosols between ferrets is warranted, in particular given the substantial public health benefits assigned to this type of research (5, 6).

PROBABILITY OF LABORATORY-ACQUIRED INFECTIONS

Initial calculations of the potential risks associated with research on influenza virus transmission via respiratory droplets or aerosols between ferrets (1–4) used reports on select agent theft, loss, and release collected by the U.S. Centers for Disease Control and Prevention (CDC) from 2004 to 2010 (7) to calculate the probability of occurrence of LAIs. Although these reports have limitations (1, 4, 7), they provide the most recent account of LAIs in the United States and probably reflect the current state of the art in biosafety and biosecurity practices better than older studies on laboratory incidents (8, 9), e.g., as a consequence of the implementation of the U.S. select agent program and best practices developed in biosafety and biosecurity in general over the last decades. From 2004 to 2010, 11 LAIs in total were reported to the U.S. CDC, 4 of which occurred in biosafety level 3 (BSL3) facilities. During this 7-year period, on average 10,000 individuals per person-year (1–4). These estimates, however, do not take into account specific pathogen types or research settings. This is crucial, because working practices in, e.g., virology and microbiology laboratories are different and because each biosafety laboratory is unique (10, 11). Research facilities and the experiments that are conducted are therefore appraised through targeted risk assessments, in which the planned studies are scrutinized before any experiment is started. On this note, it is important that none of the LAIs reported to the U.S. CDC from 2004 to 2010 involved viruses (7), and the risks of LAIs associated with work on viral pathogens should thus be estimated as less than 1 per 2,044 (<5 × 10^−4 per laboratory-year), or less than 1 per 70,000 (<1.4 × 10^−5 per person-year). Unfortunately, the report by Henkel et al. (7) does not specify how many of the 2,044 laboratory-years and 70,000 person-years were related to BSL3 facilities versus BSL2 and BSL4 facilities. Thus, using 2,044 and 70,000 as the denominators yields an underestimation of the true probability of LAIs under BSL3 conditions, as discussed previously (1, 4).

SOME KEY BIOSAFETY MEASURES AND RISK MITIGATION STRATEGIES AT ERASMUS MC

Research on influenza virus transmission via respiratory droplets or aerosols between ferrets is performed in facilities and under conditions that are specifically designed for the purpose of such studies (12–16). In ordinary BSL3 laboratories, including diagnostic laboratories, work is performed in open-front class 2 biosafety cabinets with directional airflow, aimed at protecting the environment from release of pathogens and protecting laboratory workers from exposure. Contrary to ordinary BSL3 conditions for work with viruses, all in vivo and in vitro experimental work on influenza virus transmission in the Erasmus MC facility is carried out in class 3 isolators or class 3 biosafety cabinets, which are airtight boxes with negative pressure (<−200 Pa), to ensure inward flow in case of leakage (12, 16). Handling is done through airtight gloves fitted to the front of these cabinets. Air released from the class 3 units is filtered by high efficiency particulate air (HEPA) filters and then leaves directly via the facility ventilation system, again via HEPA filters. Only authorized and experienced personnel that have received extensive training can access the facility. For animal handling, personnel always work in pairs to reduce the chance of human error. Although the laboratory is considered “clean” because all experiments are conducted in closed class 3 cabinets and isolators, special personal protective equipment, including laboratory suits, gloves, and FFP3 (class 3 filtering face piece) facemasks, are used, and all personnel are vaccinated with the homologous A/H5N1 vaccine. All equipment in the fa-
Personnel receive oseltamivir treatment upon consultation with var-
are unlikely to go unnoticed. Upon any potential exposures, per-	ronically and by visual inspection, potential exposures to virus
antibody titers decrease (12).

40) (19) and that individuals are revaccinated if and when their
/titers generally accepted as protective against seasonal influenza
antigen and treatment may have been insufficient to prevent infec-
tion altogether (hence the occurrence of the LAI at a frequency of
less than once every 1 million years), the virus shedding in H5-
vaccinated and oseltamivir-treated individuals is likely to be re-
duced substantially, compared to the onward transmission in
times of spread during an influenza pandemic from untreated
immunologically naive individuals. If we assume a conservative
2-log reduction in virus excretion in immunized and treated indi-
viduals (20–24) compared to untreated immunologically naive
individuals, the range of probability of onward transmission from
a case of LAI would be reduced to <5 × 10^{-4} to 6 × 10^{-5}.

As an important risk mitigation strategy to reduce onward trans-
mission upon any potential LAI, Erasmus MC policy dictates
enforcement of quarantine of any laboratory personnel that are
potentially virus exposed. This policy would reduce the exposure
of nonlaboratory personnel to one (the partner of the laboratory
worker) or nil, rather than the ~100 contacts human adults would
ordinarily have during a 5-day time frame (25). As a consequence,
the transmission probability can be further reduced ~100-fold,
yielding a probability of onward transmission from the case of LAI
of <5 × 10^{-6} to 6 × 10^{-5}.

A final factor to consider in the calculation of the probability of
onward transmission from each case of LAI is the basic reproduc-
tion number (R0) of the influenza virus under investigation. As
indicated above, the previous risk assessments were based on R0 of
pandemic influenza virus. However, laboratory experiments have
shown that the efficiency of transmission of the laboratory-
derived influenza viruses was lower than that of the transmission
of pandemic and seasonal influenza viruses in ferrets, as could be
expected (12, 16, 26). Moreover, given that the viruses are ferret adapted rather than human adapted, even an extremely conservative adjustment of the transmissibility parameter by a factor of 2 would yield a “final” estimation of the probability of onward transmission from a case of LAI of \(2.5 \times 10^{-6}\) to \(3 \times 10^{-5}\).

**PROBABILITY OF AN LAI FOLLOWED BY ONWARD TRANSMISSION**

Multiplying the probability of occurrence of an LAI by the probability of onward transmission from each case of LAI, one can estimate that the probability of an LAI resulting in onward transmission would range between \((1 \times 10^{-7}) \times (2.5 \times 10^{-6})\) (or \(2.5 \times 10^{-13}\)) and \((1 \times 10^{-7}) \times (3 \times 10^{-5})\) (or \(3 \times 10^{-12}\)). From this analysis, the estimate is that when research is performed on transmissible viruses, there is no consensus on the incidence of A/H5N1 infections in Southeast Asia (32, 33), but case fatality rates orders of magnitude lower than 60% have been inferred (27). In addition, it is important to note that fatalities in ferrets infected with A/H5N1 virus via respiratory droplets or aerosols have not occurred, contrary to when ferrets received large dosages of A/H5N1 virus directly in the (lower) airways (12, 13, 16).

**CONCLUDING REMARKS**

On the topic of intentional or accidental releases of viruses from laboratories involved in influenza virus transmission studies, it is important to note that during a decade of transmission studies on pandemic and epidemic strains derived from the 1918, 1957, 1968, and 2009 pandemics and on various wild-type and laboratory-adapted zoonotic viruses of subtypes H1, H2, H5, H7, and H9 (summarized in reference 16), no LAIs have been recorded. There have also been no recorded intentional or accidental releases during more than a century of research with human and animal influenza viruses, including highly pathogenic avian influenza viruses, even at times when biocontainment measures were largely nonexistent. Some have argued that the 1977 Russian influenza epidemic was the result of a laboratory accident (2), but in 1977, influenza research was done under conditions of limited biocontainment, and attenuated and wild type strains were tested in humans. We do not know what happened in 1977, but we cannot conclude that the virus escaped a BSL3(+) laboratory.

Since natural influenza pandemics have occurred on average every 30 years over the last century, the probability that the next pandemic will emerge in nature is of order of magnitude larger than emergence from a laboratory. Given the recently summarized immediate and short-term benefits of research on influenza viruses that are transmitted via respiratory droplets or aerosols between ferrets (5, 6) and the longer-term aims to fully understand and predict and prevent pandemics, combined with the extremely low risk to humans and the environment associated with properly conducted experiments, I conclude that it is sensible and acceptable to restart the research, provided that any laboratory participating in this research adopt biosafety and biosecurity conditions comparable to those that are currently in place (12–16).

**ACKNOWLEDGMENTS**

I am very grateful for the help of friends and colleagues that provided valuable input and support for this letter.

I am active in the field of research on influenza virus transmission via respiratory droplets or aerosols between ferrets, funded by the European Union and NIAID/NIH. I am an inventor on a patent related to influenza virus reverse genetics.

**REFERENCES**


